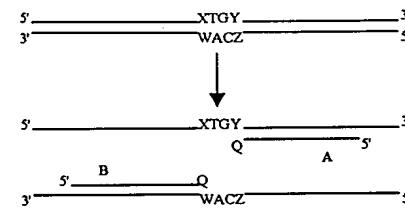
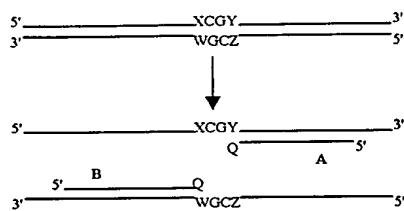


STEPS IN
PCR/RE/LDR

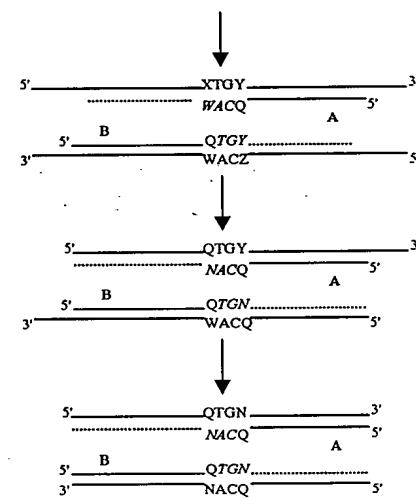
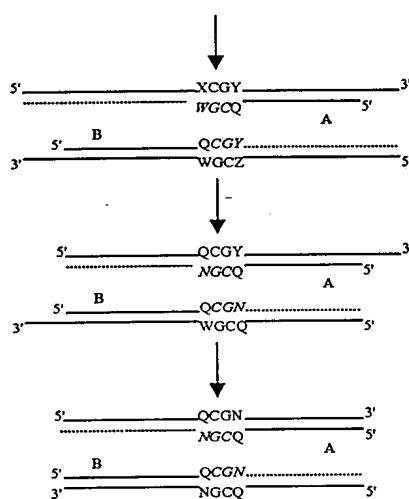
WILD-TYPE

MUTANT

1)



2)



3)

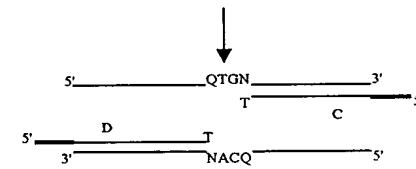
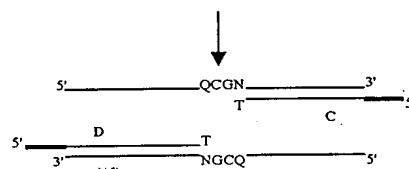
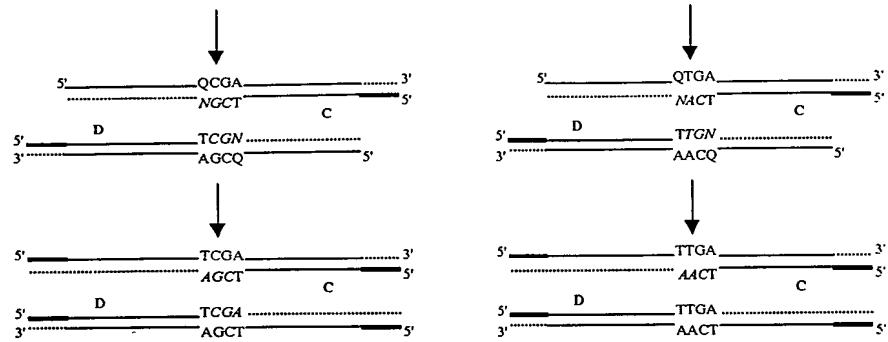


FIGURE 1

4)



5)

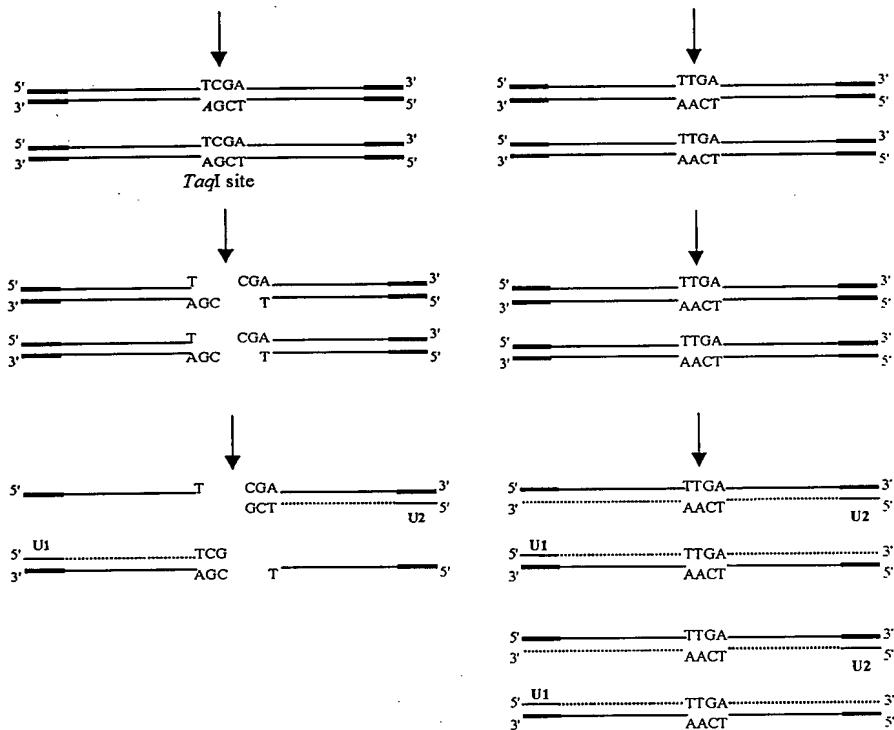
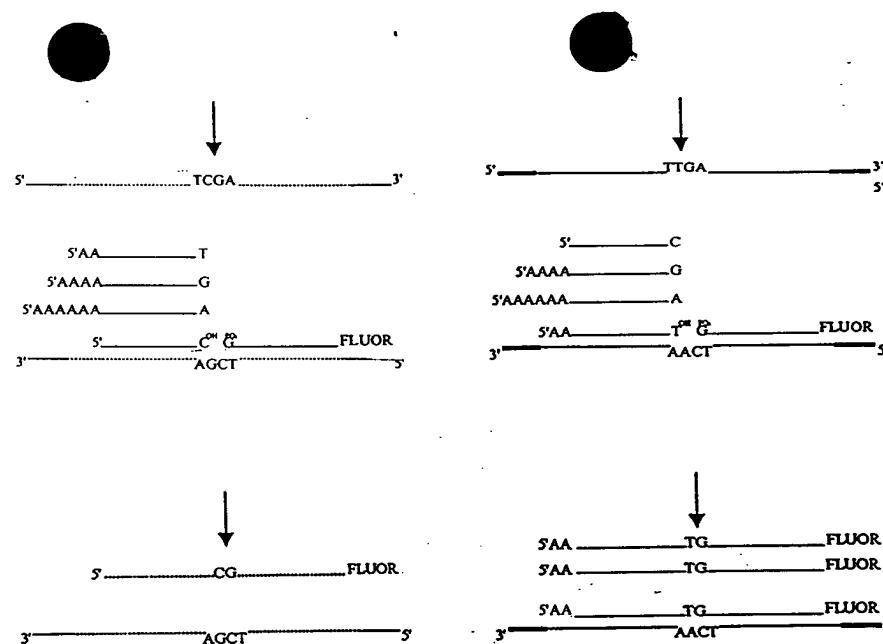


FIGURE 1 (Cont'd.)

7A)



7B)

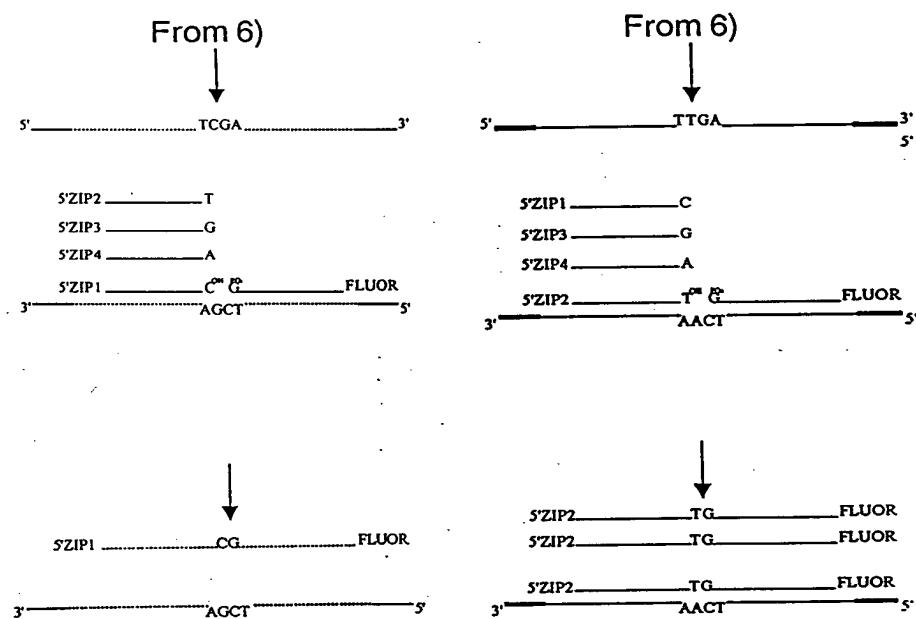


FIGURE 1 (Cont'd.)

I. GEL LANE

S'AA TG FL

S' CG FL

S'AAAAAA A

S'AAAA G

S'G FL

S'AA T

S' C

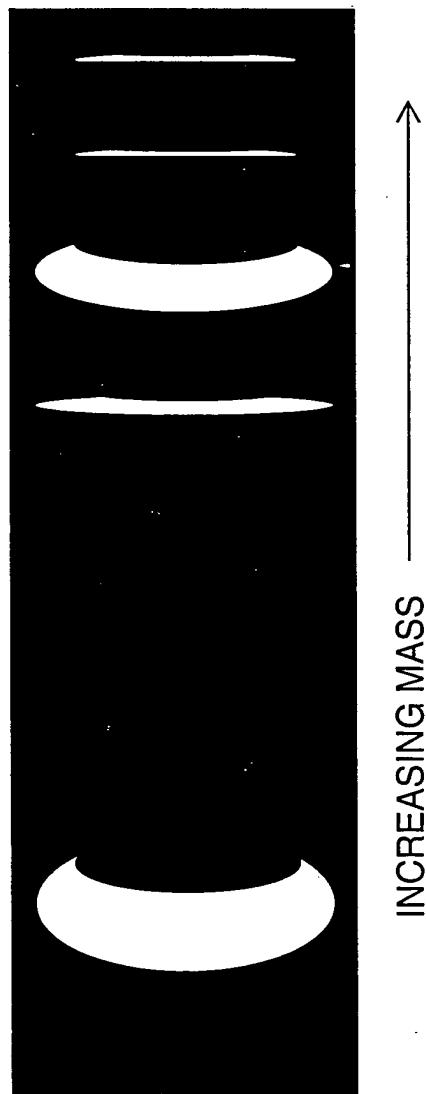


FIGURE 2

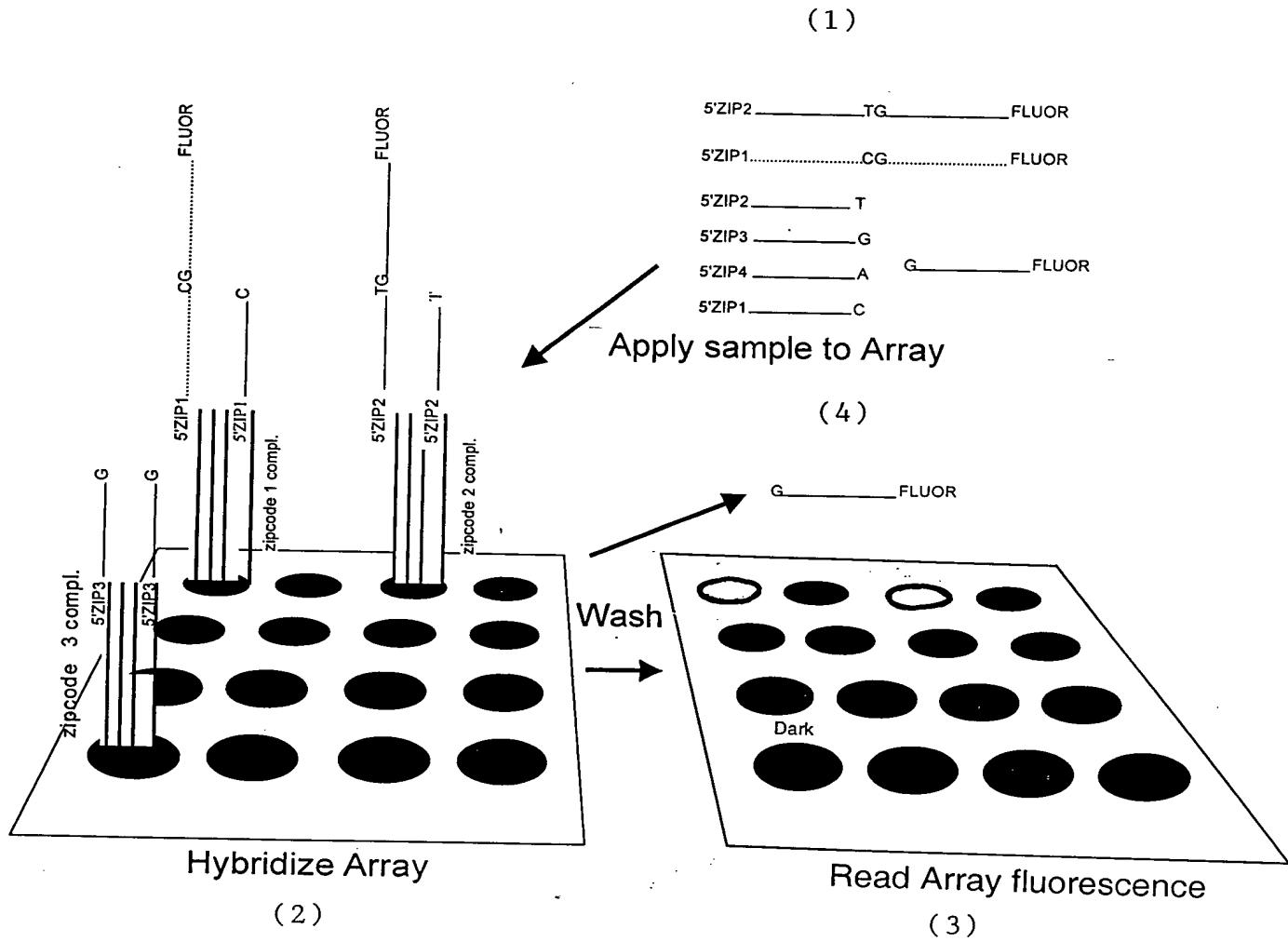


FIGURE 3

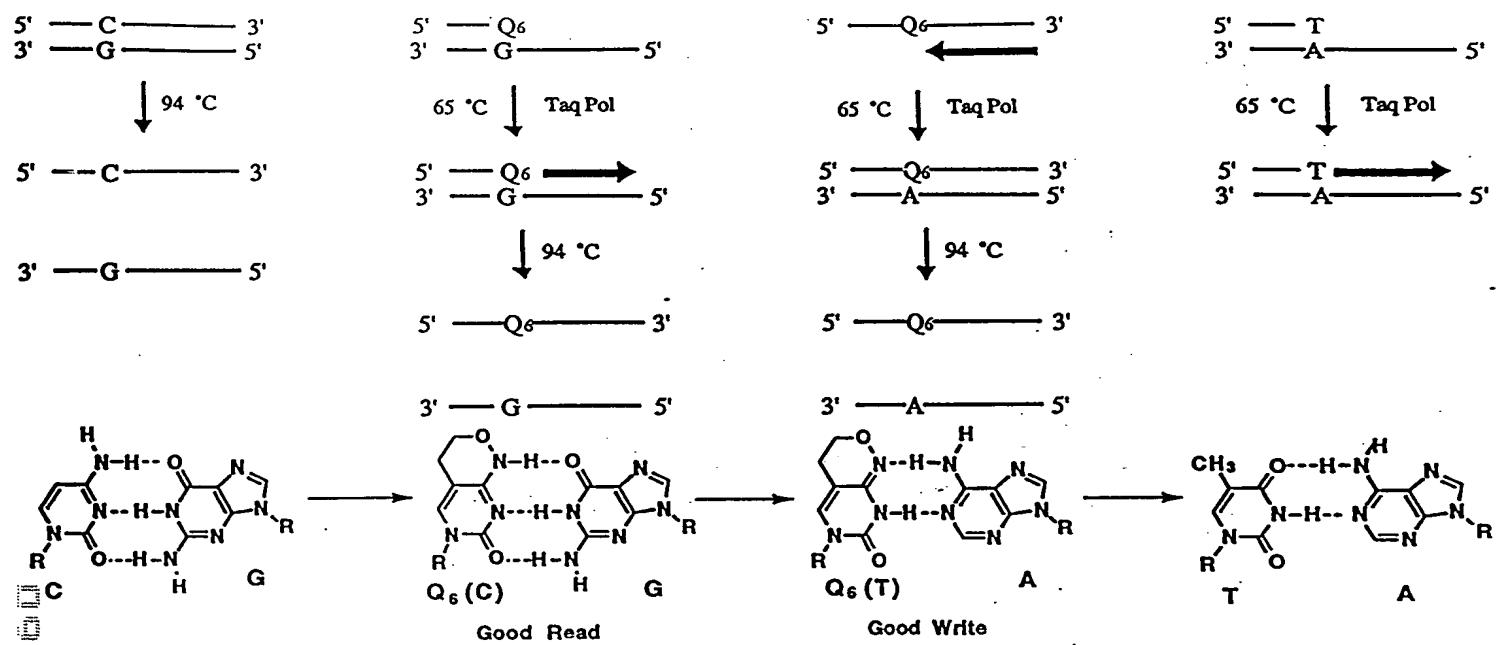
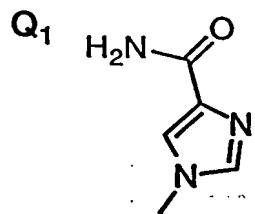
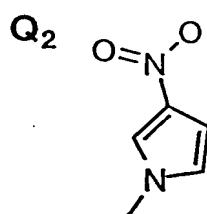


FIGURE 4

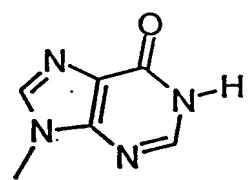
A



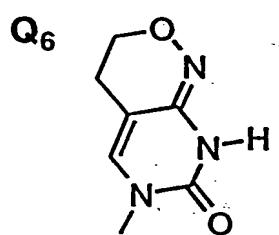
B



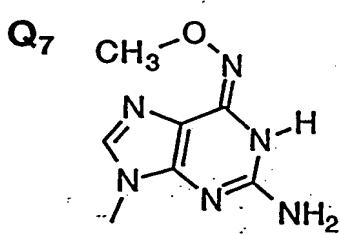
C



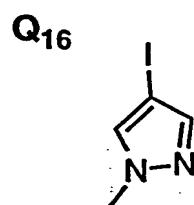
D



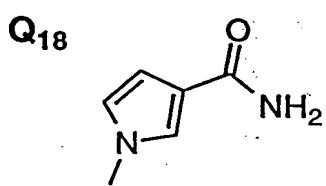
E



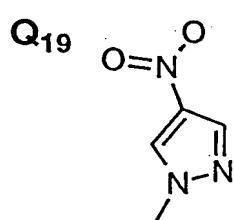
F



G



H



I

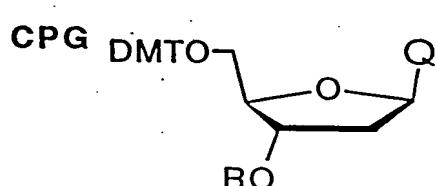


FIGURE 5

A

Primers

Ztop	CTT GGA CGA GTT CAT ACG C	↓	
p53zip248	CTT GGA CGA GTT CAT ACG CGT TCC TGC ATG GGC GGC ATG A		
p53-248X	T TCT TCC TGC ATG GGC GGC ATG AAC → pol		
50 bp synthetic duplex DNA	:		
	3' CA AGG AGC TAC CGG CGG TAC TTG GGC TCC GGG TAG GAG TGG TAG TAG TAG TGT 5' (-)		
	5' GT TCC TGC ATG GGC GGC ATG AAC GGG AGG CCC ATC CTC ACC ATC ATC ACA 3' (+)		
p53-248XR	:		
p53zip248R	pol-X TCC GGG TAG GAG TGG TAG TAG TGT T		
Zbot	C GGG TAG GAG TGG TAG TAG TCC ACC GCT GGG TCA AAC G		
	C ACC GCT GGG TCA AAC G		

B

Primers

C

LDR Primers

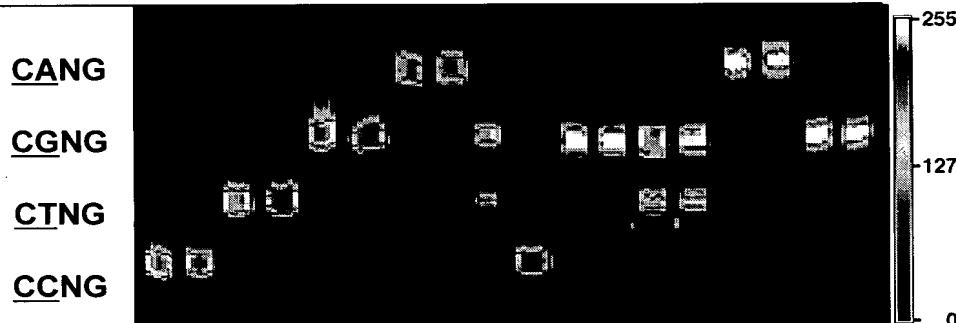
p53LDR248FCA	F-AAAAAA GC ATG GGC GGC ATG AAC A
p53LDR248FCG	F-AAAA GC ATG GGC GGC ATG AAC G
p53LDR248FCT	F-AA GC ATG GGC GGC ATG AAC T
p53LDR248FCC	F- GC ATG GGC GGC ATG AAC C
p53LDR248PGG	GG AGG CCC ATC CTC ACC ATC AT-block
conversion products	3' (-strand) ... GTC TGC GCA AGG ACG TAC CCG CCG TAC TTG ACC TCC GGG TAG GAG TGG TAG TAG TGA ACC... 5'

FIGURE 6

A

Template	CCGG	CTGG	CGGG	CAGG	TCGA	GCGC	ACGT	CATG	CGCG
Exptd prod	CCGG	CTGG	CGGG	CAGG	CCGG	CCGG	CCGG	CATG	CGCG
primer 3' end	C	Q ₆	C						
1st base	C	C	C	C	C	C	C	C	C
2nd base	C	C	T	T	G	G	G	G	G
minor 2nd					t	t	t	T	T
base prod						c	c	c	

LANE 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

**B**

Template	CCGG	CTGG	CGGG	CAGG	TCGA	GCGC	ACGT	CATG	CGCG
Exptd prod	TCGA	TTGA	TGGA	TAGA	TCGA	TCGA	TCGA	TATA	TGCA
primer 3' end	T	Q ₆	T						
1st base	T	T	T	T	T	T	T	T	T
2nd base	?	C	?	T	?	G	?	A	?
minor 2nd					g		?	c	?
base prod							g	g	

LANE 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

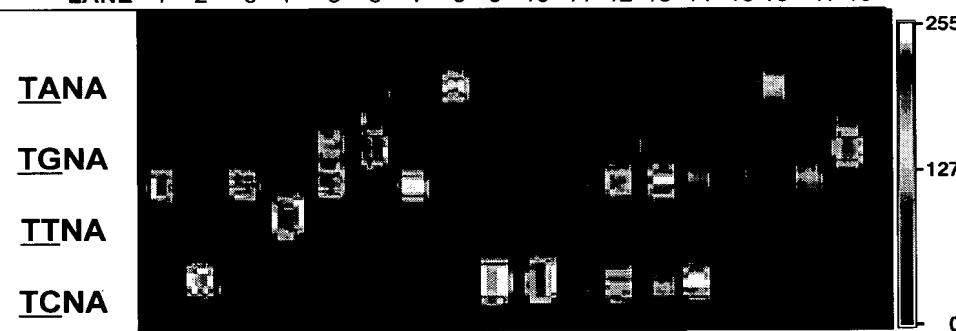


FIGURE 7

A

Template	CCGG	CTGG	CGGG	CAGG	TCGA	GCGC	ACGT	CATG	CGCG
Exptd prod	<u>GC</u> GC	<u>GT</u> GC	<u>GG</u> GC	<u>GAG</u> C	<u>GC</u> GC	<u>GC</u> GC	<u>AC</u> GT	<u>CAT</u> G	<u>CG</u> CG
primer 3' end	G Q ₅ Q ₇								
1st base	G G G	G G G	G G G	G G G	G G G	G G G	G G G	G G G	G G G
2nd base	C C C	C C C	C C C	C C C	C C C	C C C	C C C	C C C	?
minor 2nd		t	t	t	g	g	A	A	a
base prod									

LANE 1 2 4 5 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
3 6

**B**

Template	CCGG	CTGG	CGGG	CAGG	TCGA	GCGC	ACGT	CATG	CGCG
Exptd prod	<u>AC</u> GT	<u>AT</u> GT	<u>AG</u> GT	<u>AAG</u> T	<u>AC</u> GT	<u>AC</u> GT	<u>AC</u> GT	<u>AAT</u> T	<u>AG</u> CT
primer 3' end	A Q ₇ Q ₅								
1st base	A A A	A A A	A A A	A A A	A A A	A A A	A A A	A A A	A A A
2nd base	C C C	C C C	C C C	C C C	C C C	C C C	C C C	C C C	c C c
minor 2nd	?	?	?	?	?	?	?	?	?
base prod									

LANE 1 2 4 5 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
3 6

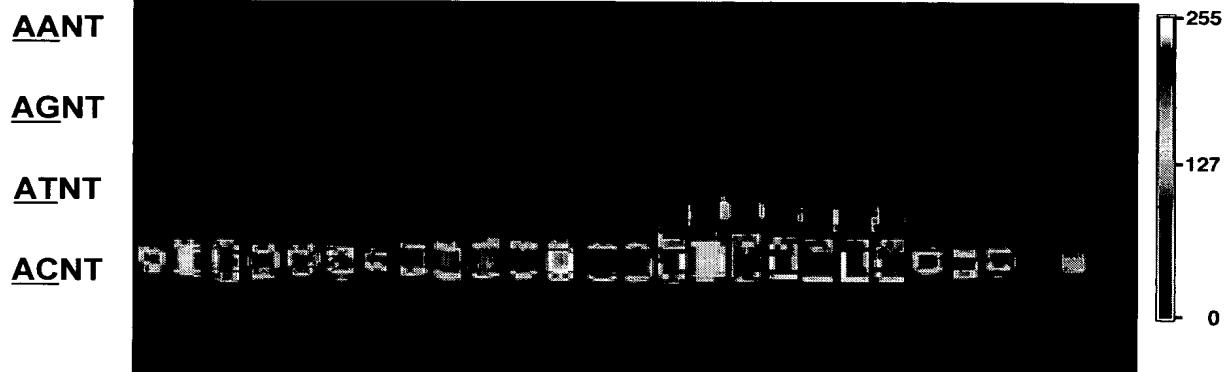


FIGURE 8

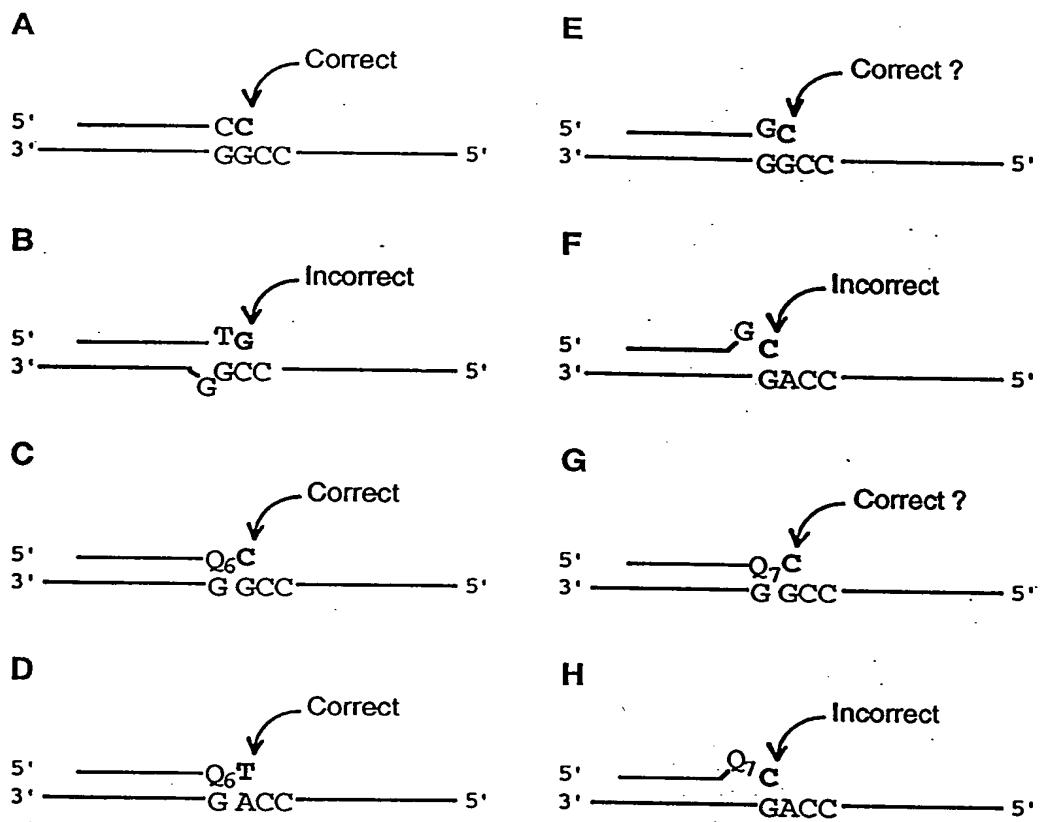


FIGURE 9

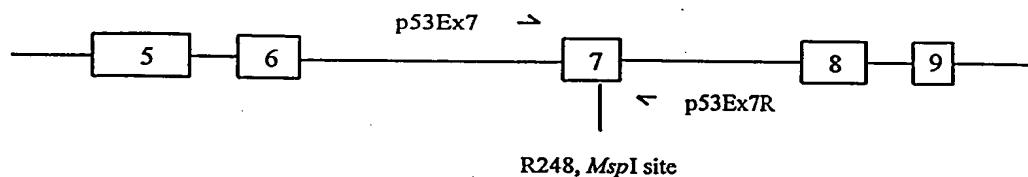
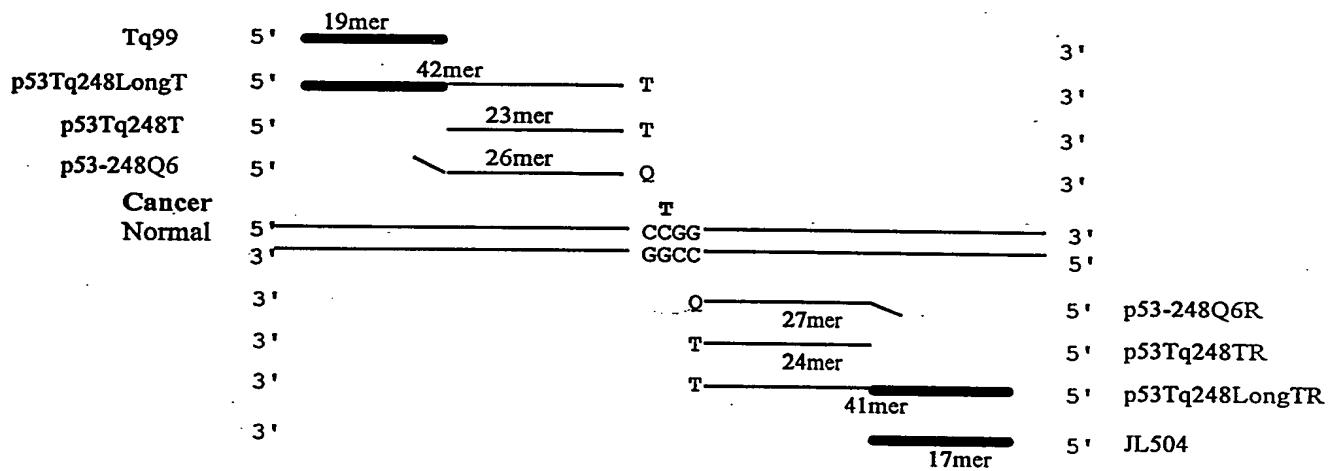
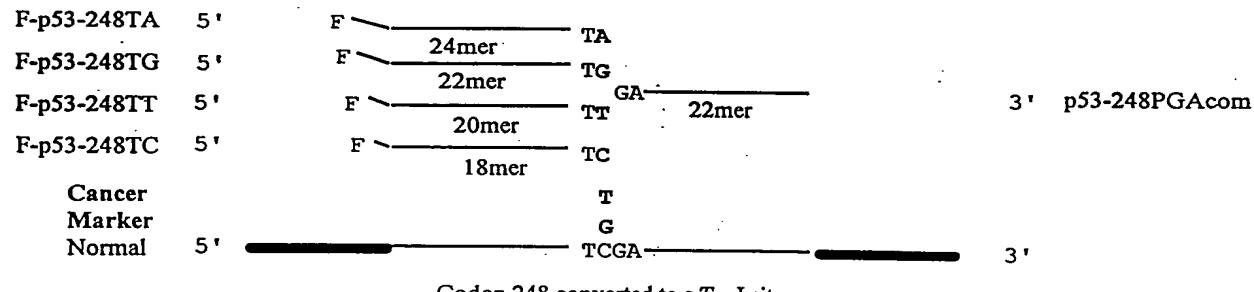
A**B****C**Codon 248 converted to a *TaqI* site

FIGURE 10

APrimers

Ztop

CTT GGA CGA GTT CAT ACG C

MspI (CCGG)

codon 248

↓

p53zip248short

CTT GGA CGA GTT CAT ACG CGT TCC TCC ATG GGC GGC ATG A

p53-248short

GT TCC TCC ATG GGC GGC A → pol
|| |||| |||| |||| |||| ||||

p53 exon 7

PCR product

(MK not shown)

p53-248shortR

p53zip248shortR

Zbot

3' ... CA AGG ACC TAC CGG CGG TAC TTG GGC TCC GGG TAG GAG TGG TAG TAG TGT ... 5' (-)

5' ... GT TCC TCC ATG GGC GGC ATG AAC GGC AGG CCC ATC CTC ACC ATC ATC ACA ... 3' (+)

pol → CG TAG GAG TGG TAG TAG TGT

C GGG TAG GAG TGG TAG TAG TGC ACC GCT GGG TCA AAC C

C ACC GCT GGG TCA AAC C

BPrimers

Ztop

CTT GGA CGA GTT CAT ACG C

MspI (CCGG)

codon 248

↓

p53zip248T

CTT GGA CGA GTT CAT ACG CGT TCC TCC ATG GGC GGC ATG AAT

GT TCC TCC ATG GGC GGC ATG AAT

p53Taq248T

p53Taq248Q₆

50-bp synthetic

duplex DNA, or

PCR product

p53Taq248Q₆R

p53Taq248TR

p53zip248TR

Zbot

3' CA AGG ACC TAC CGG CGG TAC TTG GGC TCC GGG TAG GAG TGG TAG TAG TGT 5' (-)

5' GT TCC TCC ATG GGC GGC ATG AAC GGC AGG CCC ATC CTC ACC ATC ATC ACA 3' (+)

: |||| |||| |||| |||| |||| ||||

pol → Q₆TCC GGG TAG GAG TGG TAG TAG TGT

T TCC CGG TAG GAG TGG TAG TAG TG

T TCC CGG TAG GAG TGG TAG TAG TGC ACC GCT GGG TCA AAC C

C ACC GCT GGG TCA AAC C

CLDR Primers

p53LDR248FTCL

Discrimination

Common

F-AAAAAAA GC ATG GGC GGC ATG AAT C

F-AAAAAA GC ATG GGC GGC ATG AAC A

F-AAA GC ATG GGC GGC ATG AAC G

F-AA GC ATG GGC GGC ATG AAC T

F- GC ATG GGC GGC ATG AAC C

7-ligase

GG AGG CCC ATC CTC ACC ATC AT-block

conversion

3' (-strand)

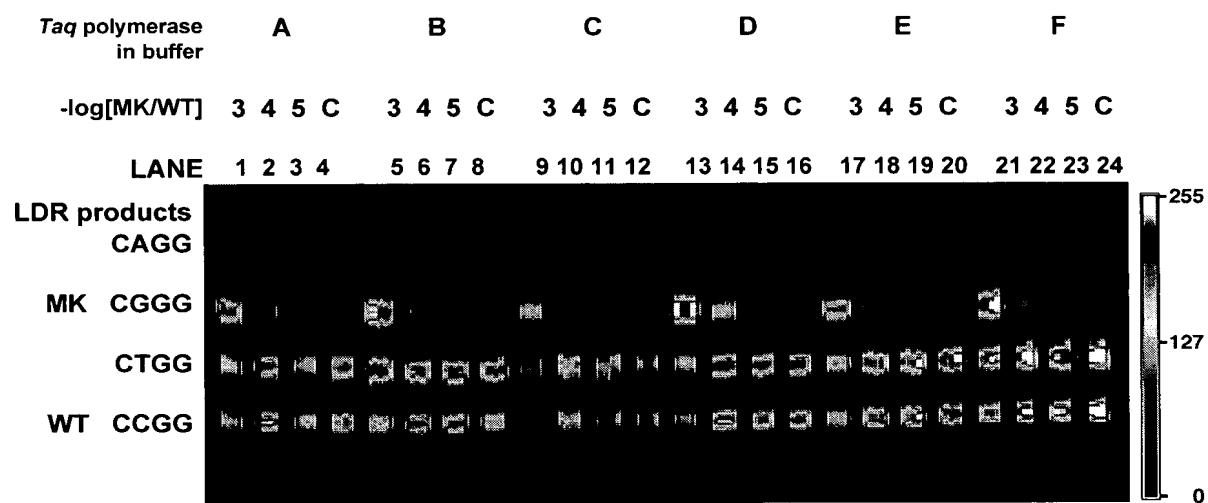
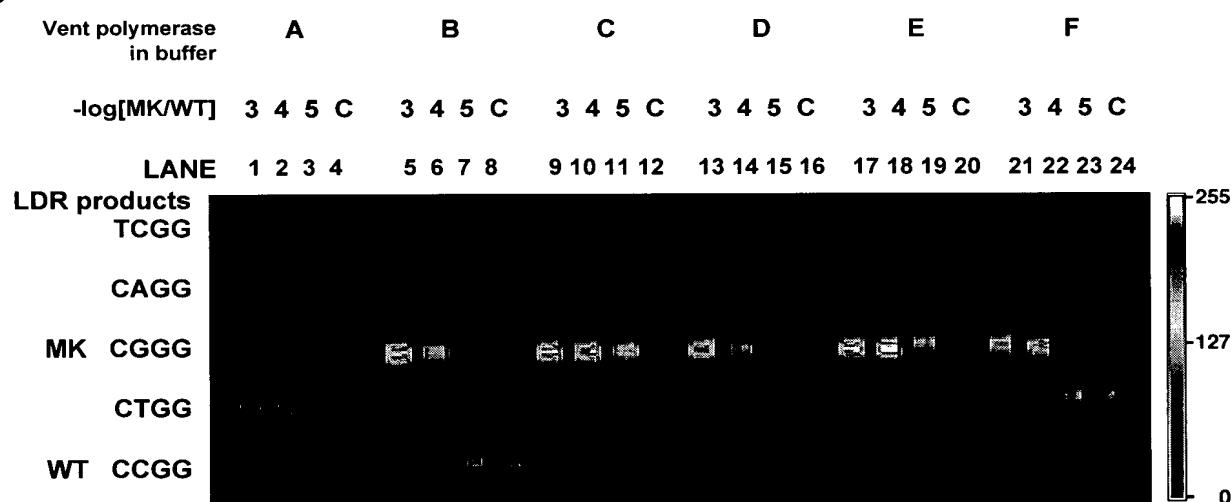
|| |||| |||| |||| |||| |||| |||| |||| |||| |||| |||| ||||

5

products

... GTC TGC GCA AGG ACG TAC CGG CGG TAC TTG NGG TCC GGG TAG GAG TGG TAG TAG TGA ACC ...

FIGURE 11

A**B****FIGURE 12**

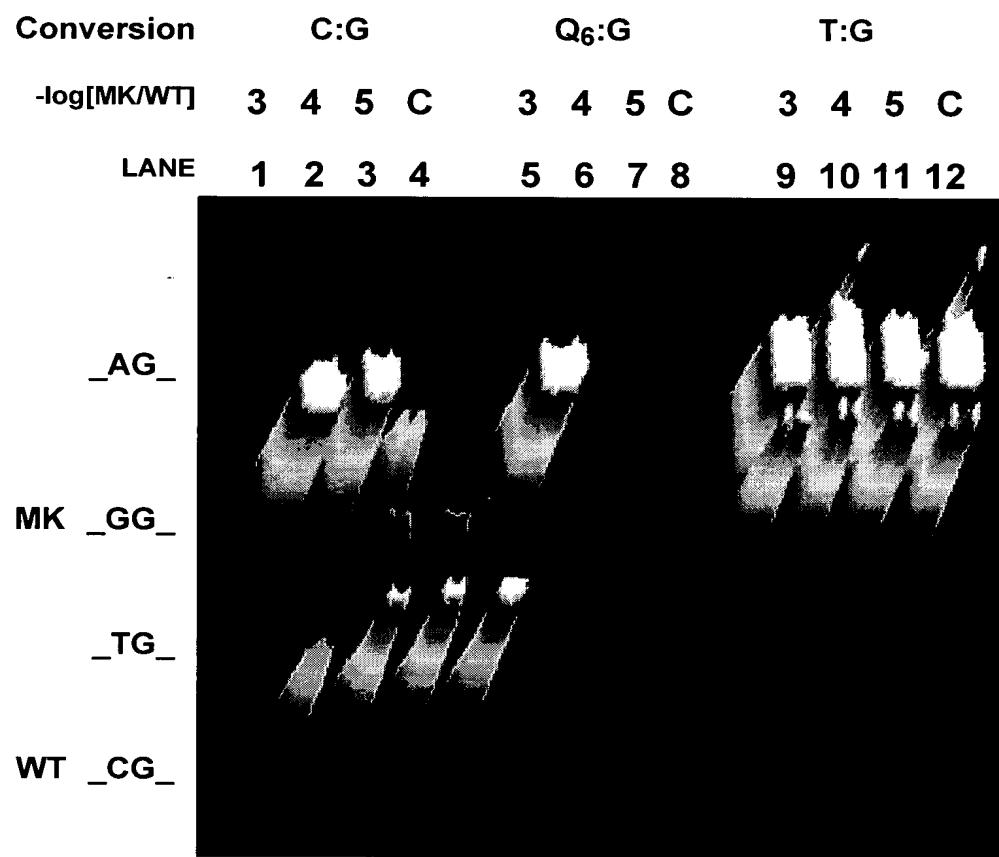


FIGURE 13